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Lorence et al., J. Natl. Cancer Inst., (1988), 80 (16), 1305-12.

Thanks

Newcastle Disease Virus as an Antineoplastic Agent: Induction of Tumor Necrosis Factor-α and Augmentation of Its Cytotoxicity[1,2,3]

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The oncolytic strain 73-T of Newcastle disease virus (NDV) has been reported to be beneficial in the treatment of cancer patients, but little is known about its mechanism of action. In this study, NDV strain 73-T and a wild-type isolate of NDV were found to be potent inducers of tumor necrosis factor (TNF) production by both human peripheral blood mononuclear cells (PBMCs) and rat splenocytes. Antibody inhibition experiments identified TNF-α as the major species of TNF induced by NDV in PBMCs. The effect of recombinant human TNF-α (rHuTNF-α) on human cancer cells was then examined. Neither rHuTNF-α nor supernatants from NDV-stimulated PBMCs were cytotoxic toward the TNF-resistant human malignant melanoma cell line MEL-14. However, when MEL-14 cells were treated with NDV strain 73-T, both rHuTNF-α and supernatants from NDV-stimulated PBMCs killed 48% and 55%, respectively, of these tumor cells. Treatment with NDV also conferred TNF susceptibility to the TNF-resistant human malignant melanoma cell line MEL-21 and the human myelogenous leukemia cell line K562. In contrast to its enhanced cytotoxicity toward NDV-treated cancer cells, rHuTNF-α had no effect on NDV-treated normal human PBMCs proliferating in response to concanavalin A. These results suggest two important mechanisms for the antineoplastic activity of NDV: (*a*) induction of TNF-α secretion by human PBMCs and (*b*) enhancement of the sensitivity of neoplastic cells to the cytolytic effects of TNF-α. [J Natl Cancer Inst 1988;80:1305-1312]

The clinical literature shows that certain viruses can destroy tumors in cancer patients (*1-5*). Such observations led to the initial wave of interest between the years 1950 and 1971 in treating cancer patients with viruses (*6-8*). However, a major drawback to this type of therapy was the observed neurotropism of the most effective oncolytic viruses, which caused encephalitis in some patients.

In an attempt to eliminate this side effect, Cassel and Garrett (*9*) used the oncolytic strain 73-T of Newcastle disease virus (NDV), which was isolated after 73 passages in vitro and 13 passages in vivo in mouse Ehrlich ascites tumor cells. This paramyxovirus caused no side effects when it was injected at high doses into humans (*9,10*). NDV strain 73-T killed some human cancer cells in vitro, caused necrosis of tumors in hamsters, and could effect a cure of mouse ascites tumors after they reached 41% of their total development (*9*). In clinical trials on humans, NDV strain 73-T replicated in the tumor of a patient with cervical cancer and reduced the size of the tumor (*9*). More recently, Cassel and co-workers (*11,12*) showed that of 32 patients with stage II malignant melanoma who were treated with an NDV oncolysate (consisting of a concentrate of live virus in tumor cell membranes), 90% remained disease free after 3 years, in contrast to <10% of control patients. In experiments with mice having a lymphoma, Heicappell et al. (*13*) and Schirrmacher et al. (*14*) recently verified that NDV and NDV-modified tumor cells can cause tumor regression and can prevent metastases.

Recent reports (*15,16*) have indicated that another paramyxovirus, Sendai virus, is a potent inducer of the monocyte/macrophage product, tumor necrosis factor (TNF), in human peripheral blood mononuclear cells (PBMCs). In light of these observations, we postulated that NDV strain 73-T would also induce secretion of TNF. TNF, also called cachectin (reviewed in refs. *17,18*), was first described by Carswell et al. (*19*) as an antitumor substance released into the sera of BCG-sensitized mice after injection with endotoxin. Since this macrophage product shares many properties with lymphotoxin, a product of mitogen- or antigen-activated lymphocytes, it has been renamed TNF-α and the lymphocyte product has been renamed TNF-β. The activities of these two cytokines include the ability to mediate cytotoxic or cytostatic effects on certain tumor lines in vitro and hemorrhagic necrosis of some tumors in vivo (*20-24*). TNF-α and TNF-β share 30% amino acid homology (*25,26*), interact at the same cell-surface receptor (*27,28*), and have genes linked to the major histocompatibility complex (*29*).

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[3]This article is dedicated to the memory of Donald Bouthillier.

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Regarding the potential use of TNF as a therapeutic agent in humans, recombinant TNF-α has been recently tested in phase I trials (*30*). Single doses of up to 200 μg of recombinant human TNF-α (rHuTNF-α)/m^2 of body-surface area were well tolerated in cancer patients (*30*). However, in animal models, the therapeutic action of TNF-α has been limited by its toxicity. Asher et al. (*31*) observed that tumor-bearing mice were more susceptible to the toxic side effects of rHuTNF-α than were normal mice. Havell et al. (*32*) found that almost lethal quantities of recombinant murine TNF-α (rMuTNF-α) were required to cause tumor regression in mice. Observations of such toxic properties are consistent with the proposed role of TNF-α in cachexia (*17*) and endotoxic shock (*33*).

Endogenous production of TNF-α at tumor sites may be one way to limit the toxic effects of TNF-α. In this article, we show that NDV strain 73-T, which has already been shown to replicate in human and murine tumors (*9*), not only is a potent inducer of TNF-α in human PBMCs but also confers sensitivity to TNF-α in human tumor cell lines that are normally resistant to the lytic effects of TNF-α. These observations indicate a role for endogenous TNF-α in the antineoplastic properties of NDV in cancer patients.

Materials and Methods

Viruses

NDV strain 73-T was obtained from Dr. William A. Cassel, Emory University, Atlanta, GA. A wild-type isolate of NDV was a gift from Dr. Deoki N. Tripathy, University of Illinois, Urbana, IL. These strains of NDV were grown in the allantoic cavity fluid of 11-day fertilized chicken eggs, harvested after 2 days, and quantified in hemagglutination units (HAU) (*34*).

Tumor Cells

The human myelogenous leukemia cell line K562 was provided by Dr. David Kranz, University of Illinois, Urbana, IL. The human malignant melanoma cell lines MEL-14 and MEL-21 were gifts from Dr. Michael J. Walker and Dr. Tapas Das Gupta, University of Illinois, Chicago, IL. The murine fibrosarcoma cell line WEHI 164 (clone 13) was obtained from Dr. Terje Espevik, Genentech, Inc., South San Francisco, CA. The murine transformed fibroblast line L929 (ATCC CCL 1) was provided by the American Type Culture Collection, Rockville, MD.

Recombinant TNF and Antibodies

The following products were gifts from Dr. H. Michael Shepard of Genentech, Inc.: rHuTNF-α, 5.0×10^7 U/mg; recombinant human TNF-β (rHuTNF-β), 1.2×10^8 U/mg; rMuTNF-α, 2.9×10^7 U/mg; affinity-purified monoclonal antibody against rHuTNF-α from mouse ascites, 6,000 neutralizing units/μg of protein; and affinity-purified rabbit antiserum to rHuTNF-β, 2.9×10^7 neutralizing units/mL. One unit of TNF has been defined as the reciprocal of the dilution of a preparation that results in 50% killing of dactinomycin (Act D)-treated L929 cells (*35*).

Interferons

Interferons (IFNs) that were tested in the TNF assay were purified cell-derived murine IFN-α (Lee Biomolecular, San Diego, CA) and recombinant rat IFN-γ(Amgen, Thousand Oaks, CA).

TNF Assay

TNF was detected by a bioassay according to the procedure of Espevik and Nissen-Meyer (*36*), with minor modifications. WEHI 164 clone 13 target cells were seeded in 96-well microplates (Falcon Microtest III plates; Becton, Dickinson & Co., Oxnard, CA) at a concentration of 4×10^4 cells/well in 100 μL of medium RPMI-1640 (Sigma Chemical Co., St. Louis, MO) containing 10% heat-inactivated fetal bovine serum (FBS), 100 U of penicillin/mL, and 100 μg of streptomycin/mL. After the cells were incubated overnight at 39 °C in 7% CO_2 in a humidified chamber, the medium was removed and replaced with 50 μL/well of RPMI-1640 containing 10% FBS and 1.33 μg of Act D/mL (Sigma Chemical Co.). Test samples of 50 μL in RPMI-1640 containing 10% FBS were then added to each well. After the cells were incubated for an additional 18 hours, a cytotoxicity assay was performed with the use of the organic dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co.) according to the methods of Mosmann (*37*), with minor modifications. The medium was removed from each well and replaced with 50 μL of 1 mg of MTT/mL in 0.85% saline. After the cells were incubated at 39 °C for 4 hours, the MTT-containing solution was removed (while not disturbing any of the blue tetrazolium crystals within the cells) and replaced with 50 μL of 0.015 *M* phosphate-buffered saline (PBS) (pH 7.2) and 100 μL of acid-isopropanol (0.04 *N* HCl in isopropanol) per well. After the blue crystals were dissolved by repeated pipetting, the absorbance at 540 nm was determined by using a Bio-Tek model 310 enzyme-linked immunosorbent assay reader (Bio-Tek Instruments Inc., Burlington, VT). Cells that were lysed with 0.01% Triton X-100 (TX-100) (Sigma Chemical Co.) served as a measurement of 100% cytotoxicity. Percent specific cytotoxicity due to a sample containing TNF was determined by the formula: percent specific cytotoxicity = 100 × {[absorbance (cells + medium) − absorbance (cells + sample)]/[absorbance (cells + medium) − absorbance (cells + TX-100)]}.

Human PBMC and Rat Splenocyte Preparations

Six milliliters of heparinized (100 U/mL) whole blood, collected from healthy male volunteers between 25 and 35 years of age, was added to 6.7 mL of Sepracell-MN (Sepratech Corp., Oklahoma City, OK). After being mixed by gentle inversion, the tubes were centrifuged at 2,000 *g* at room temperature. The PBMC layer was removed and washed once with 15 mL of 0.015 *M* PBS containing 0.1% bovine serum albumin (BSA), hereafter referred to as PBS/BSA, at pH 7.2. Cells were pelleted by centrifugation at 300 *g* for 10 minutes at room temperature. Erythrocytes in the PBMC pellet were lysed by resuspending the pellet in 0.5 mL of 0.83% NH_4Cl in 0.1% $KHCO_3$–0.01 m*M* EDTA at 4 °C for 3 min-

utes. PBMCs were washed an additional three times with PBS/BSA and resuspended at 10^6 cells/mL in RPMI-1640 without serum.

Wistar-Furth rats were housed and cared for in accordance with University of Illinois guidelines. Single-cell suspensions of splenocytes were prepared from 3-month-old female rats (*38*). Erythrocytes were lysed as described above, and splenocytes were resuspended in RPMI-1640 without serum.

TNF Induction

PBMCs, (10^6 cells/mL) and rat splenocytes (3×10^6 cells/mL) were incubated for 24 hours at 39 °C in 7% CO_2 with NDV and control egg allantoic fluid at various concentrations. Supernatants were obtained by centrifuging the medium at 4,000 *g* at 4 °C for 5 minutes. Supernatants were then heated at 60 °C for 30 minutes to kill any remaining virus, as described by Berent et al. (*16*), before dilution in RPMI-1640 and assaying for TNF. Such heat inactivation of the virus does not affect TNF activity (*16*).

Cytotoxicity Assay of Human Cancer Cells

The nonadherent K562 cells were incubated for 3 hours at a concentration of 4×10^6 cells/mL in RPMI-1640 containing 10% FBS with 100 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL). Labeled cells were then washed three times with 20 mL of RPMI-1640 before resuspension at 4×10^5 cells/mL in RPMI-1640 containing 10% FBS. One hundred microliters of this cell suspension was added to each well in a 96-well microtiter plate. Cells in each well were treated by addition of medium, NDV, or control egg allantoic fluid diluted in 50 μL of RPMI-1640 containing 10% FBS. After 2 hours of incubation with NDV, TNF samples in 50 μL of RPMI-1640 containing 10% FBS were added to each well and incubated for 18 hours. After the microtiter plate was centrifuged at 400 *g* for 10 minutes at room temperature, 100 μL of supernatant was removed from each well, mixed with 3 mL of Aquasol-2 liquid scintillation cocktail (DuPont/NEN Products, Boston, MA), and counted in a Beckman LS-5801 scintillation counter. Cells were treated with 0.1% TX-100 to determine maximal release of ^{51}Cr. Percent specific cytotoxicity was calculated from the following formula: percent specific cytotoxicity = 100 × [(sample counts per minute − spontaneous counts per minute)/(maximum release counts per minute − spontaneous counts per minute)].

Adherent MEL-14 and MEL-21 cells were plated in a 96-well microtiter plate at a concentration of 4×10^4 cells/well in 100 μL of Hanks' balanced salt solution (Sigma Chemical Co.), 10% FBS, and 1 μCi of ^{51}Cr. After the cells were incubated overnight at 39 °C, radioactive medium was removed and each well was washed three times with 200 μL of RPMI-1640 before addition of 100 μL of medium and 50 μL of NDV or control medium. After a 2-hour treatment period, control TNF samples or supernatants (100 μL) to be tested for TNF were added to each well. Following an incubation of 18 hours at 39 °C with 7% CO_2, 100 μL of medium from each well was counted and the percent cytotoxicity was determined as above. Spontaneous release of ^{51}Cr never exceeded 25% of the maximum release for any of the target cells that were used in these experiments. Evidence that K562, MEL-14, and MEL-21 cells were indeed infected with NDV was based on cytopathic changes that were observed microscopically in some cells 3 days after addition of NDV.

Effects of NDV and TNF on Lectin-Induced Proliferation of PBMCs

Cytotoxic effects of NDV and TNF on human PBMCs proliferating in response to 5 μg of concanavalin A (Con A)/mL were measured by use of the ^{51}Cr procedures described for the nonadherent K562 cells.

The cytostatic effects of NDV and TNF on human PBMCs proliferating in response to Con A were also determined. PBMCs at 5×10^6 cells/mL were added in 100-μL aliquots to microtiter wells. Two days after the addition of 100 μL of Con A (final concentration of 5 μg/mL), cells were incubated with or without 10 HAU of NDV and 1 μCi of [*methyl*-^3H]thymidine (6.7 Ci/mmol). Two hours later, aliquots of various TNF concentrations were added, and the samples were incubated for an additional 16 hours. Cells were then harvested onto glass-fiber disks and dried. Finally, 3 mL of a toluene-omnifluor (DuPont/NEN) scintillation cocktail was added, and radioactivity was measured in a Beckman LS 5801 liquid scintillation counter.

Antibody Inhibition Studies

Specific antibodies (23 neutralizing units) against either rHuTNF-α, rHuTNF-β, or control medium without antibodies were mixed with either 1 U of rHuTNF-α, 1 U of rHuTNF-β, or 10 μL of supernatant from NDV-stimulated human PBMCs. After incubation at 39 °C for 2 hours, samples (in 30 μL) were tested for TNF activity in the TNF assay.

Results

Sensitivity of WEHI 164 Clone 13 Cells to Picogram Quantities of rHuTNF-α and rHuTNF-β

In table 1 a standard method of measuring cytotoxicity using ^{51}Cr release of labeled WEHI 164 clone 13 targets was compared with a more recent and sensitive cytotoxicity assay (*37*) based on viability determination using the dye MTT. Both assays yielded similar results (table 1). Treatment of WEHI 164 clone 13 cells with the RNA-synthesis inhibitor Act D resulted in a slight increase in the percent cytotoxicity caused by either rHuTNF-α or rHuTNF-β. This effect is well illustrated with 1 U of rHuTNF-α, which had a 37% specific cytotoxicity toward WEHI 164 clone 13 cells in the absence of Act D and a 73% specific cytotoxicity in its presence. Similar results were obtained with rHuTNF-β. Under the various assay conditions tested (table 1), WEHI 164 clone 13 cells were sensitive to 0.1 U of TNF, which corresponded to 2 pg of rHuTNF-α and 0.8 pg of rHuTNF-β. These results are similar to those of Espevik and Nissen-Meyer (*36*).

Table 1. Sensitivity of murine fibrosarcoma WEHI 164 clone 13 cells to cytotoxic effects of rHuTNF-α and rHuTNF-β*

Amount of TNF (U)	% specific cytotoxicity				
	rHuTNF-α			rHuTNF-β	
	^{51}Cr release†	MTT without Act D‡	MTT with Act D‡	MTT without Act D	MTT with Act D
0	0 ± 1	0 ± 4	0 ± 4	0 ± 0	0 ± 7
0.01	6 ± 1	1 ± 1	1 ± 5	7 ± 8	6 ± 9
0.1	32 ± 3	34 ± 5	49 ± 3	28 ± 1	43 ± 3
1	48 ± 1	37 ± 4	73 ± 3	57 ± 1	91 ± 2
10	69 ± 6	78 ± 2	99 ± 1	90 ± 1	91 ± 2
25	89 ± 4	ND	ND	ND	ND
100	ND	97 ± 1	103 ± 1	100 ± 1	100 ± 1

*Values = means ± SEM of at least three different assays. ND = not determined.

†Percent specific cytotoxicity was measured by ^{51}Cr release of labeled cells in an 8-hr assay.

‡Percent specific cytotoxicity was measured by using an MTT-cell viability assay after cells were incubated with TNF samples for 18 hr with or without 0.67 μg of Act D/mL.

Lack of Cytotoxicity of Cell-Derived Murine IFN-α and Recombinant Rat IFN-γ Toward WEHI 164 Clone 13 Cells

While rHuTNF-α, rHuTNF-β, and rMuTNF-α were all highly toxic toward WEHI 164 clone 13 cells, the IFNs that were tested at much higher concentrations (murine IFN-α and recombinant rat IFN-γ) had negligible cytotoxicity toward these target cells (table 2). Therefore, these cytokines are not expected to interfere in the detection of TNF-α or TNF-β with the use of the WEHI 164 clone 13 cells.

Cytotoxicity of Heat-Inactivated Supernatants of NDV-Treated Human PBMCs and NDV-Treated Rat Splenocytes Toward WEHI 164 Clone 13 Cells and Transformed Murine L929 Fibroblasts

As sections B and C of table 3 show, heat-inactivated supernatants from NDV-stimulated human PBMCs and from NDV-stimulated rat splenocytes were cytotoxic toward WEHI 164 clone 13 cells. For example, supernatants (1:5,000 dilution) from NDV strain 73-T-stimulated human PBMCs (10^6 cells/mL) killed 48% of the WEHI 164 clone 13 cells. Supernatants (1:667 dilutions) from NDV strain 73-T-stimulated rat splenocytes (10^6 cells/mL) killed 100% of the WEHI 164 clone 13 cells. Supernatants (1:667 dilution) from rat splenocytes (10^6 cells/mL) stimulated with wild-type NDV also induced significant cytotoxicity (49%) in these target cells.

Table 2. Lack of cytotoxity of cell-derived murine IFN-α and recombinant rat IFN-γ toward murine fibrosarcoma WEHI 164 clone 13 cells

Cytokine	Amount (pg)	% specific cytotoxicity*
rHuTNF-α	100	99 ± 1
rHuTNF-β	100	91 ± 2
rMuTNF-α	100	90 ± 3
Recombinant rat IFN-γ	5,000	1 ± 3
Murine IFN-α	7,000	3 ± 5

*Values = means ± SEM of three assays. Percent specific cytotoxicity was measured by using an MTT-cell viability assay after cells were incubated with cytokine samples for 18 hr with 0.67 μg of Act D/mL.

Table 3. Cytotoxicity of heat-inactivated supernatants of NDV-treated human PBMCs and NDV-treated rat splenocytes toward murine fibrosarcoma WEHI 164 clone 13 cells and murine transformed L929 fibroblasts*

Treatment	% specific cytotoxicity	
	WEHI 164 clone 13†	L929‡
A. rHuTNF-α (U)		
0	0 ± 3	0 ± 5
1	77 ± 3	2 ± 4
10	96 ± 1	7 ± 4
100	100 ± 1	12 ± 5
B. Supernatants from human PBMCs treated with§		
Medium	7 ± 6	1 ± 5
400 HAU of NDV strain 73-T	48 ± 5	4 ± 1
Control egg fluid	2 ± 5	5 ± 2
C. Supernatants from rat splenocytes treated with§		
Medium	5 ± 3	1 ± 1
200 HAU of NDV strain 73-T	100 ± 2	6 ± 2
500 HAU of NDV strain 73-T	99 ± 2	6 ± 2
200 HAU of wild-type NDV	49 ± 3	3 ± 1
Control egg fluid	2 ± 9	3 ± 2
D. Control medium treated with¶		
Medium	0 ± 5	0 ± 1
500 HAU of NDV strain 73-T	3 ± 6	1 ± 3
500 HAU of wild-type NDV	3 ± 5	1 ± 6

*Values = means ± SEM of three assays (section A), supernatants from three human volunteers (section B) or three rats (section C), and three control cultures (section D).

†Percent specific cytotoxicity was measured by using an MTT-cell viability assay with 0.67 μg of Act D/mL.

‡Percent specific cytotoxicity was measured by using an MTT-cell viability assay without Act D.

§All supernatants were heated at 60 °C for 30 min and added at a dilution of 1:5,000 (human PBMCs) or 1:667 (rat splenocytes) to the WEHI 164 clone 13 cells. Both human and rat supernatants were used at a dilution of 1:667 in the assay on L929 cells.

¶Control medium (RPMI-1640 with 10% FBS) was treated with NDV, heated at 60 °C for 30 min, and added at a dilution of 1:667 to both types of target cells.

To exclude the possibility that some of the cytotoxic activity in the supernatants was due to direct effects of NDV on the target cells, all supernatants were heated at 60 °C for 30 minutes. TNF remains stable under these conditions (*16*). As section D of table 3 shows, heat-inactivated NDV had no direct cytotoxic effect on WEHI 164 clone 13 cells.

Although L929 cells cultured in the presence of Act D are commonly used as a bioassay for TNF activity, L929 cells cultured without Act D were much less sensitive to TNF compared with the sensitivity of WEHI 164 clone 13 cells (table 3, section A). For example, 1 U of rHuTNF-α killed 77% of the WEHI 164 clone 13 cells, while it had no cytotoxic effect on L929 cells cultivated without Act D. Therefore, by using these two cell lines, we were able to determine whether NDV induces accumulation of other toxic products, such as reactive oxygen intermediates, from PBMCs and rat splenocytes that might nonspecifically kill cells. A 1:5,000 dilution of supernatants from NDV-treated human PBMCs and a 1:667 dilution of NDV-treated rat

splenocytes were cytotoxic toward murine WEHI 164 clone 13 cells but not toward proliferating murine L929 cells. These results support the conclusion that NDV induces both human PBMCs and rat splenocytes to secrete TNF, and this protein, rather than other toxic products, is responsible for killing the target WEHI 164 clone 13 cells.

Based on the assumption of a linear relationship between percent cytotoxicity and the amount of rHuTNF between 0.1 and 10 U (tables 1, 3), 419 U and 295 U of TNF were estimated to be produced by 10^6 human PBMCs and 10^6 rat splenocytes, respectively, upon stimulation with NDV (table 4). Production of TNF resulting from control egg allantoic fluid was negligible.

Identification by Specific Antibodies of TNF-α as Major Species of TNF Induced by NDV in Human PBMCs

As table 5 shows, an affinity-purified monoclonal antibody against rHuTNF-α inhibited 91% of the activity of rHuTNF-α, whereas it did not affect the activity of rHuTNF-β. Similarly, an affinity-purified polyclonal antibody against rHuTNF-β inhibited 99% of the activity of rHuTNF-β, but it did not affect the activity of rHuTNF-α.

These specific antibodies against the two species of TNF were used to investigate TNF activity induced by NDV in human PBMC supernatants. Nearly all of the activity in supernatants from NDV-treated human PBMCs was inhibited by antibodies against rHuTNF-α (98% inhibition), whereas only a small amount of activity was inhibited by antibodies against rHuTNF-β (12% inhibition; table 5). These experiments identified TNF-α as the major species of TNF induced by NDV in human PBMCs.

Sensitivity to rHuTNF-α Conferred by Treatment of TNF-Resistant Human Cancer Cells With NDV

Untreated human malignant melanoma MEL-14 cells were totally resistant up to 2,500 U of rHuTNF-α (table 6). However, upon exposure to NDV strain 73-T, the MEL-14 cells became remarkably sensitive to killing by TNF. One hundred units and 2,500 U of rHuTNF-α killed 21% and 48%, respectively, of the NDV-infected MEL-14 cells. Similarly, heat-inactivated supernatants from NDV-treated human PBMCs displayed enhanced cytotoxic effects toward the NDV-infected MEL-14 cells (55% vs. 8%). NDV alone had no cytotoxic effect on these cancer cells. This finding supports the conclusion that TNF-α was present in the supernatants from NDV-treated human PBMCs.

When another human malignant melanoma cell line (MEL-21) was used, rHuTNF-α had no effect on the untreated cells (table 7). NDV alone killed 17% of the MEL-21 cells, but addition of 100 U of rHuTNF-α enhanced this cytotoxic effect fourfold (67%). Less dramatic but significant results were noted for human myelogenous leukemia K562 cells (table 7). Again, rHuTNF-α was not cytotoxic toward the untreated cells. NDV alone killed 20% of the cells, and this cytotoxicity was enhanced almost twofold upon addition of rHuTNF-α to the NDV-treated cells (34%). TNF-α was

Table 5. Use of specific antibodies against rHuTNF-α and rHuTNF-β to identify TNF-α as major species of TNF induced by NDV in human PBMCs

Treatment	% specific cytoxicity toward WEHI 164 clone 13 cells		
	No antibody	Anti-rHuTNF-α	Anti-rHuTNF-β
rHuTNF-α, 1 U	82 ± 1	7 ± 1 (91)	90 ± 1 (−10)
rHuTNF-β, 1 U	47 ± 1	53 ± 1 (−12)	1 ± 1 (99)
Supernatant from NDV-stimulated human PBMCs (1:125 dilution)	98 ± 1	2 ± 2 (98)	86 ± 2 (12)

*Values = means ± SEM for three assays, each with rHuTNF-α and rHuTNF-β and supernatants of PBMCs from two human volunteers. Percent inhibition is given in parentheses. Percent specific cytotoxicity was measured by using an MTT-cell viability assay with 0.67 μg of Act D/mL. Antibody inhibition experiments were performed as described in Materials and Methods section.

Table 4. Estimation of amount of TNF induced in human PBMCs and rat splenocytes by NDV strain 73-T

Cells	Treatment	TNF production (U/10^6 cells)*
Human PBMCs	Medium	46 ± 41
	Control egg fluid	<30
	NDV strain 73-T†	419 ± 47
Rat splenocytes	Medium	<30
	Control egg fluid	<30
	NDV strain 73-T‡	295 ± 2

*Values = means ± SEM of supernatants of cells from three human volunteers or three rats. Procedure for estimation of TNF production is described in Materials and Methods section.

†Four hundred hemagglutination units of NDV strain 73-T added per 10^6 cells.

‡One hundred and sixty-seven hemagglutination units of NDV strain 73-T added per 10^6 cells.

Table 6. Cytotoxicity of rHuTNF-α and supernatants from NDV-stimulated human PBMCs toward NDV-infected but not toward uninfected malignant melanoma MEL-14 cells*

NDV infection of MEL-14 cells	Treatment	% specific cytotoxicity toward MEL-14 cells†
−	Medium	0 ± 1
−	rHuTNF-α, 100 U	0 ± 3
−	rHuTNF-α, 2,500 U	4 ± 1
−	NDV-stimulated PBMC supernatant	8 ± 4
+	Medium	0 ± 1
+	rHuTNF-α, 100 U	21 ± 5
+	rHuTNF-α, 2,500 U	48 ± 7
+	NDV-stimulated PBMC supernatant	55 ± 7

*Values = means ± SEM of three assays of supernatants of PBMCs that were uninfected (−) or infected (+) with 10 HAU of NDV. PBMCs were obtained from two human volunteers. All supernatants were heat treated at 60 °C for 30 min before being added to ^{51}Cr-labeled MEL-14 cells.

†Percent specific cytotoxicity was determined after 18 hr of treatment with the use of a ^{51}Cr release assay described in Materials and Methods section.

Table 7. Effects of rHuTNF-α and NDV treatment on human malignant melanoma MEL-21 cells, human myelogenous leukemia K562 cells, and human PBMCs proliferating in response to Con A

NDV added (HAU)	rHuTNF-α (U)	% specific cytotoxicity*		
		MEL-21	K562	PBMCs
0	0	0 ± 8	0 ± 1	0 ± 0
0	100	3 ± 6	2 ± 1	4 ± 4
10	0	17 ± 4	20 ± 1	3 ± 2
10	100	67 ± 1	34 ± 1	2 ± 2

*Values = means ± SEM of two different assays on each type of cell. Percent specific cytotoxicity was determined after 18 hr of treatment with the use of a ^{51}Cr assay described in Materials and Methods section.

demonstrated to be the cytolytic molecule, because antibodies against rHuTNF-α blocked 100% of the cytolytic activity of rHuTNF-α against NDV-treated tumor cells.[6]

Sensitivity to Lytic Effects of TNF-α Not Induced by Treatment of Normal Proliferating Cells With NDV

In these experiments, proliferating human PBMCs were used rather than unstimulated PBMCs, since the former cells would serve as a better comparison with proliferating tumor cells. In contrast to its enhanced cytotoxicity toward NDV-treated cancer cells, rHuTNF-α did not kill NDV-treated human PBMCs proliferating in response to Con A (table 7). Similarly, neither NDV (10 HAU), rHuTNF-α (up to 1,000 U), nor the combination of these two reagents affected the capability of normal human PBMCs during the last 18 hours to proliferate in response to Con A (data not shown). As a positive control, Act D (1 μg/mL) was added during the last 18 hours of the proliferation assay. Unlike NDV, Act D totally inhibited the uptake of ^{3}H by PBMCs (stimulation index, <1), even though it was not cytotoxic to these cells (specific ^{51}Cr release, 8%).

Discussion

In this article we investigated one aspect of the antineoplastic properties of a paramyxovirus. We focused our attention on NDV, since it has been administered in live form to cancer patients for the past 20 years with claims of beneficial results and minimal side effects (*5,9,11,12*). In our experiments, two major observations were made regarding the mechanisms by which NDV can induce lysis of tumor cells: (*a*) NDV elicits TNF-α production by human PBMCs and (*b*) TNF-α can kill NDV-treated, TNF-resistant human cancer cells but not normal proliferating human PBMCs treated with NDV. Furthermore, in marked contrast to the nonspecific effects of many cancer chemotherapeutic agents such as Act D, the combination of NDV and TNF that killed up to 67% of malignant melanoma MEL-21 cells (table 7) did not inhibit proliferation of Con A-stimulated PBMCs. These results strongly suggest an important role for TNF in some of the antineoplastic properties of NDV.

Various approaches were used to reach the conclusion that NDV is capable of inducing TNF in human PBMCs. First, it was necessary to determine if any IFN might have activity in the TNF bioassay, since NDV is a potent inducer of IFN-α (*10*). The WEHI 164 clone 13 cell line employed in the TNF bioassay was sensitive to 2 pg of TNF-α, whereas it was insensitive to 5,000 pg of the IFNs (murine IFN-α and rat IFN-γ, table 2). Supernatants from both human and rat mononuclear cells treated with NDV, but not supernatants from untreated mononuclear cells, could lyse the sensitive WEHI 164 clone 13 cells. However, these supernatants at the same dilution had no effect on the L929 cell line that is much less sensitive to the lytic effects of TNF-α. All of the lytic activity in the supernatants of NDV-treated human PBMCs could be abrogated by treatment with a specific antibody to TNF-α, but not with an antibody to TNF-β. Furthermore, three different human transformed cell lines that are resistant to the lytic effects of TNF-α could be rendered susceptible to killing by TNF-α after they were exposed to NDV.

Perhaps the most important result reported in this article is the observation that an agent (NDV) that is administered to cancer patients is a potent inducer of TNF in vitro. Levels of TNF induced by NDV reported here (400 U/10^6 PBMCs) are comparable with those induced by another paramyxovirus, Sendai virus [150 U/10^6 human PBMCs (*15*)]. Two lines of evidence also suggest that TNF is produced in vivo during viral infections. First, macrophages often predominate at sites of viral infection (*39*), and macrophages are a major cellular source of TNF-α. Second, Mak et al. (*40*) and Chapes and Tompkins (*41*) have shown that rodents infected with influenza A, Sendai, and vaccinia viruses generate macrophages that are cytotoxic toward virally infected cells but not toward uninfected cells. Since TNF-α is a potent mediator of macrophage/monocyte cytotoxicity (*42,43*), in vivo induction of TNF during viral infections seems likely.

Virus enhancement of the cytotoxic activity of TNF-α and of TNF-β occurs after exposure to vesicular stomatitis virus (*44,45*), adenovirus type 2 (*46*), and herpesvirus (*47*). Eifel et al. (*44*) postulated that this effect is due to the viral inhibition of host protein synthesis that is normally needed to overcome TNF-mediated cytotoxicity. It is well known that infection with many viruses can potently inhibit host RNA and protein synthesis (*48*). Furthermore, inhibition of protein synthesis with agents like cycloheximide confers TNF sensitivity to TNF-resistant cells (*49–51*). Strong additional evidence for a common mechanism by which viruses and protein-synthesis inhibitors augment TNF cytotoxicity is the finding that a protease inhibitor reverses the effect of both cycloheximide (*51*) and paramyxoviruses.[6] Highly relevant to our findings are the observations of Fransen et al. (*23*), who showed that a very diverse set of human cancer cells could be made dramatically more sensitive to the cytotoxicity of TNF in the presence of the RNA-synthesis inhibitor Act D. If enhancement of TNF cytotoxicity due to viral exposure occurs by the same mechanism as that induced by RNA- or protein-synthesis inhibitors, the recent work of Fransen et al. (*23*) would suggest that our observations of virus-enhanced

[6]Rood PA, Lorence RM, Kelley KW: manuscript in preparation.

TNF cytotoxicity toward MEL-14, MEL-21, and K562 cells would extend to many different types of human cancer cells. The necessity of a live virus to enhance TNF cytotoxicity toward tumor cells was demonstrated by comparing TNF cytotoxic activity toward tumor cells treated with live NDV with that toward tumor cells treated with UV radiation-inactivated NDV. The UV treatment of NDV abrogated up to 80% of TNF-α activity for NDV-treated tumor cells.[6]

An attempt to elicit in vivo production of TNF by injecting NDV into mice was unsuccessful (data not shown), presumably because an active viral infection was needed to provide a more continuous stimulus for macrophage production of TNF. NDV has not been shown to replicate in adult mice except in those with tumors (*9*). Indeed, NDV strain 73-T was first isolated after 73 passages in mouse Ehrlich ascites tumor cells (*9*), which probably enhanced its tumor specificity and reduced its neurovirulence. Therefore, a logical extension of our results would be to test for possible synergistic effects when both NDV strain 73-T and TNF-α are administered in vivo to tumor-bearing rodents.

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E6            1     NEWCOL 1000SN/CN
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E9            1     NEWCOL 1120/CN
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=> s ("73-t" or 73t or 73 t) and (ndv or (newcastle or new
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TOTAL FOR ALL FILES
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L14            4 FILE BIOSIS
L15            3 FILE MEDLINE
L16            2 FILE EMBASE
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L19           11 MESOGENIC (W)(NEWCASTLE OR NEW CASTLE)(W) DISEASE VIRUS

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L10  ANSWER 1 OF 6  BIOSIS  COPYRIGHT 1996 BIOSIS      DUPLICATE 1
AN  95:412268  BIOSIS
TI  Can Virus Therapy of Human Cancer Be Improved by Apoptosis
    Induction?.
SO  Medical Hypotheses 44 (5). 1995.  359-368. ISSN: 0306-9877
AU  Sinkovics J G; Horvath J
AB  Direct virus inoculations and viral oncolysates may induce temporary
    remissions and prolong life with reduced tumor burden, or decrease
    relapse rates but fall short of curing human cancers. We propose: (i)
    investigations of Cassel's 73-T, an Ehrlich's
    mouse ascites carcinoma-adapted Newcastle disease
    virus (NDV) strain that so effectively reduced relapse
    rates in malignant melanoma if it is an admixture or a recombinant
    with a murine parvovirus; (ii) transfection of prostatic carcinoma
    cells with the TRMP gene; (iii) transfection of sarcoma cells with
    the fas gene followed by treatment with anti-fas monoclonal
    antibodies, and (iv) treatment of metastatic tumors with a parvovirus
    incorporating the apoptosis-inducer Ad5 E1A gene. Thus, replicating
    virions and haphazard generation of cytokines in the inoculated host
    could be replaced with transfection of single genes of well-defined,
    limited but selected efficacy.

L10  ANSWER 2 OF 6  CAPLUS  COPYRIGHT 1996 ACS         DUPLICATE 2
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AN 1995:240920 CAPLUS
TI Complete regression of human fibrosarcoma xenografts after local
n **wcastl diseas** virus therapy
SO Cancer Res. (1994), 54(23), 6017-21
CODEN: CNREA8; ISSN: 0008-5472
AU Lorence, Robert M.; Katubig, Burt B.; Reichard, Kirk W.; Reyes, Hernan M.; Phuangsab, Anan; Sassetti, Mark D.; Walter, Robert J.; Peeples, Mark E.
PY 1994
AB We have recently demonstrated a single local injection of the avian pathogen **Newcastle disease** virus (**NDV**; strain **73-T**) causes complete regression of human neuroblastoma xenografts in athymic mice (R. M. Lorence, K. W. Reichard, B. B. Katubig, H. M. Reyes, A. Phuangsab, B. R. Mitchell, C. J. Cascino, R. J. Walter, and M. E. Peeples. J. Natl. Cancer Inst., 86: 1228-1233, 1994). In this report, we tried to det. if this in vivo antineoplastic effect of **NDV** extends to human sarcomas. Athymic mice with s.c. HT1080 fibrosarcoma xenografts (7-13 mm) were randomly divided into two groups and treated i.t. with a single injection of either 107 divided into two groups and treated i.t. with a single injection of either 107 plaque-forming units of **NDV** or phosphate-buffered saline. Complete tumor regression occurred in 8 of 10 mice treated with **NDV** while unabated tumor growth occurred in all 9 mice treated with
NDV while unabated tumor growth occurred in all 9 mice treated with phosphate-buffered saline ($P < 0.001$). To det. if compelte tumor regresion was long lasting, the 8 mice were monitored for 1 yr, during which time no tumor recurred. To test the antitumor effects of **NDV** on tumors derived from a fresh human sarcoma, a similar expt. was performed in athymic mice using TH15145 synovial sarcoma xenografts at their first and second passages. Of 9 mice with TH15145 xenografts, a single i.t. injection of **NDV** (107 plaque-forming units) caused complete regression of 3 rumors and >80% regression in 3 more tumors. In contrast, tumors in all 5 mice treated with phosphate-buffered saline exhibited unabated growth ($P < 0.03$ for <80% tumor regression). Since HT1080 fibrosarcoma cells express the N-ras oncogene, we expolred the effects that transfection of this oncogene has on the sensitivity to **NDV**. Cultured human fibroblasts that were made tumorigenic following N-ras-transfection were found in a cytotoxicity assay. Oncogene expression by the HT1080 fibrosarcoma may therefore contribute to the long-lasting complete regression of this sarcoma following a single local injection of **NDV**.

L10 ANSWER 3 OF 6 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 3
AN 94:452347 BIOSIS
TI Complete regression of human neuroblastoma xenografts in athymic mice after local **Newcastle disease** virus therapy.
SO Journal of the National Cancer Institute (Bethesda) 86 (16). 1994. 1228-1233. ISSN: 0027-8874
AU Lorence R M; Reichard K W; Katubig B B; Reyes H M; Phuangsab A; Mitchell B R; Cascino C J; Walter R J; Peeples M E
AB Background: Neuroblastoma is the most common pediatric extra-cranial solid cancer. Using conventional therapies, children older than 1

year of age with advanced neuroblastoma have a poor prognosis. The development of new approaches for treating such children with neuroblastoma continues to be one of the most important goals today in pediatric oncology. Despite numerous anecdotal reports of human tumor regression during viral infections, the use of viruses to directly lyse neuroblastoma cells has never been reported as a potential therapy. **New-castle disease** virus (**NDV**) has been shown to replicate in and kill cultured human and rat neuroblastoma cells but not normal human fibroblasts. Purpose: Our purpose was to determine if this selective killing of human neuroblastoma (IMR-32) cells is maintained during the in vivo treatment of established tumors. Methods: Two experiments were performed using **NDV** strain **73-T**. Athymic mice with subcutaneous IMR-32 human neuroblastoma xenografts (6-12 mm) were treated intralesionally with live **NDV**, UV-inactivated **NDV**, or phosphate-buffered saline (PBS). To study virus replication in situ, mice were given intratumoral or intramuscular injections of **NDV**. These mice were then killed at various times, and the amount of infectious virus present in tumor or muscle was determined. Results: After one injection of live **NDV**, 17 of 18 tumors regressed completely, whereas rapid tumor growth occurred in all 18 mice treated with PBS and in all nine mice treated with UV-inactivated **NDV** (P lt .0001). The one tumor that showed only a partial response to a single injection regressed completely after a second **NDV** treatment. Six months following virus-induced regression, only one tumor had recurred. No significant acute or chronic side effects of live **NDV** were noted in athymic mice given doses up to 500 times that used in this study. Virus levels increased more than 80-fold between 5 and 24 hours in virus-injected tumors (P lt .04), while no infectious virus was produced in **NDV**-injected muscle tissue. Conclusions: **NDV 73-T** appears to replicate selectively in human IMR-32 neuroblastoma xenografts, leading directly to a potent antitumor effect as demonstrated by long-lasting complete tumor regression occurring after a single local injection of virus. Implication: These experiments may provide an important step in the development of new therapeutic approaches to challenging cancers such as neuroblastoma.

L10 ANSWER 4 OF 6 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 4
AN 94:535364 BIOSIS
TI Induction of cytokines and cytotoxicity against tumor cells by **Newcastle disease** virus.
SO Cancer Biotherapy 9 (3). 1994. 225-235. ISSN: 1062-8401
AU Zorn U; Dallmann I; Grosse J; Kirchner H; Poliwoda H; Atzpodien J
AB The use of **NDV** as biological adjuvant in vaccines against human cancer is still actual in several clinical treatment protocols. In this study, we have investigated in vitro-effects of **Newcastle disease** virus (**NDV**) strain **73-T** on isolated mononuclear blood cells and cultured tumor cells. Cellular cytotoxicity of PBMC freshly isolated from healthy donors against tumor cells was enhanced significantly (p lt 0.01) after coincubation of **NDV** with effector cells. **NDV** failed to enhance cytotoxicity of effector cells when PBMC were stimulated three days with 500 IU recombinant interleukin-2

(rIL-2) per ml prior to coincubation with the virus. No significant enhancement of cellular lysis was seen when only target cells were coincubated with **NDV**. As shown by depletion of various lymphocyte subsets, NK cells were the predominant mediator of lysis. Enhancement of cytotoxicity correlated with the induction of interferon-alpha (IFN-alpha) in PBMC by **NDV**. **NDV** also induced high amounts of tumor necrosis factor-alpha (TNF-alpha) in PBMC. Induction of interferon-gamma (IFN-gamma) was weak. A direct cytopathic effect (CPE) of **NDV** on different target cells was detected by colorimetric measurement of metabolic cell activity. The human tumor cell lines A-498, A-704, Caki-1, Caki-2, and K-562 and the fibroblast line MRC-5 showed progressive cellular destruction 48h after infection with **NDV**, whereas PBMC and Daudi cells remained unaffected during the observation period. The nontransformed monkey kidney cell line CV-1 and the transformed monkey kidney cell line COS-1 were both lysed by **NDV** with marginal difference in time course of CPE. Our results indicate a reasonable potential of pleiotropic modifications of the immune response against tumors by **NDV**.

L10 ANSWER 5 OF 6 MEDLINE DUPLICATE 5
AN 92318632 MEDLINE
TI **Newcastle disease** virus selectively kills human tumor cells.
SO JOURNAL OF SURGICAL RESEARCH, (1992 May) 52 (5) 448-53. Journal code: K7B. ISSN: 0022-4804.
AU Reichard K W; Lorence R M; Cascino C J; Peeples M E; Walter R J; Fernando M B; Reyes H M; Greager J A
PY 1992
AB **Newcastle disease** virus (**NDV**), strain **73-T**, has previously been shown to be cytolytic to mouse tumor cells. In this study, we have evaluated the ability of **NDV** to replicate in and kill human tumor cells in culture and in athymic mice. Plaque assays were used to determine the cytolytic activity of **NDV** on six human tumor cell lines, fibrosarcoma (HT1080), osteosarcoma (KHOS), cervical carcinoma (KB8-5-11), bladder carcinoma (HCV29T), neuroblastoma (IMR32), and Wilm's tumor (G104), and on nine different normal human fibroblast lines. **NDV** formed plaques on all tumor cells tested as well as on chick embryo cells (CEC), the native host for **NDV**. Plaques did not form on any of the normal fibroblast lines. To detect **NDV** replication, virus yield assays were performed which measured virus particles in infected cell culture supernatants. Virus yield increased 10,000-fold within 24 hr in tumor and CEC supernatants. Titers remained near zero in normal fibroblast supernatants. In vivo tumoricidal activity was evaluated in athymic nude Balb-c mice by subcutaneous injection of 9 x 10(6) tumor cells followed by intralesional injection of either live or heat-killed **NDV** (1.0 x 10(6) plaque forming units [PFU]), or medium. After live **NDV** treatment, tumor regression occurred in 10 out of 11 mice bearing KB8-5-11 tumors, 8 out of 8 with HT-1080 tumors, and 6 out of 7 with IMR-32 tumors. After treatment with heat-killed **NDV** no regression occurred (P less than 0.01, Fisher's exact test). Nontumor-bearing mice injected with 1.0 x 10(8) PFU of **NDV** remained healthy. These

results indicate that **NDV** efficiently and selectively replicates in and kills tumor cells, but not normal cells, and that intralesional **NDV** causes complete tumor regression in athymic mice with a high therapeutic index.

L10 ANSWER 6 OF 6 CA COPYRIGHT 1996 ACS DUPLICATE 6
AN 109:228341 CA
TI **Newcastle disease** virus as an antineoplastic agent: induction of tumor necrosis factor-.alpha. and augmentation of its cytotoxicity
SO J. Natl. Cancer Inst. (1988), 80(16), 1305-12
CODEN: JNCIEQ; ISSN: 0027-8874
AU Lorence, Robert M.; Rood, Pamela A.; Kelley, Keith W.
PY 1988
AB The oncolytic strain **73-T** of **Newcastle disease** virus (**NDV**) has been reported to be beneficial in the treatment of cancer patients, but little is known about its mechanism of action. **NDV** strain **73-T** and a wild-type isolate of **NDV** were found to be potent inducers of tumor necrosis factor (TNF) prodn. by both human peripheral blood mononuclear cells (PBMCs) and rat splenocytes. Antibody inhibition expts. identified TNF-.alpha. as the major species of TNF induced by **NDV** in PBMCs. Neither rHuTNF-.alpha. nor supernatants from **NDV**-stimulated PBMCs were cytotoxic toward the TNF-resistant human malignant melanoma cell line MEL-14. However, when MEL-14 cells were treated with **NDV** strain **73-T**, both rHuTNF-.alpha. and supernatants from **NDV**-stimulated PBMCs killed 48% and 55%, resp., of these tumor cells. Treatment with **NDV** also confered TNF susceptibility to the TNF-resistant human malignant melanoma cell line MEL-21 and the human myelogenous leukemia cell line K562. These results suggest two important mechanisms for the antineoplastic activity of **NDV**: (a) induction of TNF-.alpha. secretion by human PBMCs and (b) enhancement of the sensitivity of neoplastic cells to the cytolytic effects of TNF-.alpha..

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L20 6 DUP REM L19 (5 DUPLICATES REMOVED)

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L20 ANSWER 1 OF 6 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 1
AN 95:300374 BIOSIS
TI Brain lesions in chickens experimentally infected with a neuroadapted strain of **mesogenic Newcastle disease virus.**
SO Journal of Veterinary Medical Science 57 (2). 1995. 237-244. ISSN: 0916-7250
AU Bhaiyat M I; Kobayashi Y; Itakura C; Islam M A; Kida H
AB Neuroadapted Newcastle disease virus (Q-10) was selected by tenth serial passage, in the chicken brain, of a mesogenic strain (Q-0) originally isolated from quails. Specific pathogen-free birds were inoculated intranasally with one of these viruses. At daily intervals

for 7 days and then at 10, 14, and 21 days post-inoculation (PI), two birds from each group were killed and samples of the brain were collected for histopathological and virological examination. Q-10 caused severe nonsuppurative encephalitis with nervous signs and high mortality. Lesions characterized by neuronal degeneration and necrosis, perivascular lymphocytic infiltration, and focal or diffuse astrogliosis occurred mainly in the parahippocampal cortex, hippocampus, hyperstriatum, neostriatum, subleptomeningeal and periventricular regions of the cerebrum. Spongy changes with neuronal degeneration and axonal spheroids were also observed in the brain stem of a few cases. The amount of virus in the brain reached a peak on day 4 PI and virus could not be recovered from the brain after 6 days PI. In contrast, Q-0 caused nonfatal asymptomatic disease and virus could not be isolated from the brain, sections of which showed only minimal inflammatory changes. This difference in the lesions of the brain might be related to neurovirulence and, neuroadaptation by serial passage may occur by increased efficiency of viral replication in neurons.

L20 ANSWER 2 OF 6 BIOSIS COPYRIGHT 1996 BIOSIS
AN 94:315396 BIOSIS
TI Use of BHK-21-adapted **mesogenic Newcastle disease virus** for primary vaccination of chicks.
SO Indian Journal of Animal Sciences 64 (5). 1994. 436-438. ISSN: 0367-8318
AU Kumanan K; Vijayarani K; Parthiban M; Padmanaban V D
AB The use of BHK-21-adapted mesogenic 'Komarov' strain of Newcastle disease virus for primary vaccination of chicks was studied. The efficacy of the vaccine was assessed by haemagglutination-inhibiting antibody titres and challenge experiments. The BHK-21-adapted virus was safe and potent for priming the chicks by oculonasal route. Further studies are in progress.

L20 ANSWER 3 OF 6 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 2
AN 84:179404 BIOSIS
TI VIRUS ISOLATIONS FROM PET BIRDS SUBMITTED FOR IMPORTATION INTO THE USA.
SO AVIAN DIS 27 (3). 1983. 731-744. CODEN: AVDIAI ISSN: 0005-2086
AU SENNE D A; PEARSON J E; MILLER L D; GUSTAFSON G A
AB From October 1973-September 1981, 2,882,111 birds were offered for importation into the USA. All were quarantined for 30 days; specimens were submitted to the laboratory for virus isolation studies. Viruses were isolated from specimens from 26.3% (598/2274) of the quarantined lots of birds. Viscerotropic velogenic Newcastle disease virus (VVNDV) was isolated from 141 lots. Nonviscerotropic velogenic Newcastle disease virus (VNDV) was isolated from 6 lots. All VVNDV- and VNDV-positive lots were refused entry. The percentage of lots refused entry declined from 31.6% in 1974 to 2.9% in 1981. **Mesogenic Newcastle disease virus** (NDV) was isolated from 3 lots, lentogenic NDV was isolated from 23 lots. Lots positive for mesogenic and lentogenic NDV were allowed to enter the USA. Hemagglutinating (HA) viruses other than NDV were isolated from 24.5% (373/1521) of the lots imported through privately owned quarantine facilities. Of the 8563 HA viruses isolated, 1558 were selected for identification. Forty-six percent were identified

as paramyxovirus type 2 [PMV-2], 34% were PMV-3 and 20% were influenza A viruses possessing the hemagglutinin subtypes H3, H4, H7 and H10 and the neuraminidase subtypes N1, N6, N7 and N8. The frequency of PMV-2 and PMV-3 isolations fluctuated from year to year; the frequency of isolations of influenza A viruses decreased from 64% in 1974 to 0.2% in 1981. Viruses that did not agglutinate chicken red blood cells were isolated from 52 lots. Psittacine herpesvirus (Pacheco's disease) was isolated from 25 lots of psittacines. Viruses identified by EM as reoviruses were isolated from 24 lots. Two isolations of poxvirus and 1 isolation of an unidentified adenovirus were obtained from parrots from 3 lots.

L20 ANSWER 4 OF 6 EMBASE COPYRIGHT 1996 ELSEVIER SCI. B.V.
AN 81236062 EMBASE
TI Defense mechanism against Newcastle disease virus in chicken maxillary sinus.
SO MIE MED. J., (1980) 30/2 (155-166).
CODEN: MMJJAI
AU Nozaki S.
PY 1980
AB The mechanism of resistance to **mesogenic Newcastle disease virus** infection in the chicken maxillary sinus and turbinate by intranasal inoculation was studied. The lesions induced by NDV-B following intranasal inoculation usually produced a selective destruction of significant portions of the inner surface of the turbinate scroll, but sinus lesions though sometimes present in NDV infection were not common. These results were supported by the amount of virus and the mucociliary transport time in turbinate and sinus. The chicken maxillary sinus was infected by intrasinus inoculation with NDV-B. Although cessation of the mucociliary function was not observed by the application of 5% cocain into the maxillary sinus, the deceleration of the sinus clearance time after treatment was statistically significant. Virus was recovered from the chicken maxillary sinuses which were treated with 5% cocain after 24 hours of intranasal NDV infection, and this is supported by the histologic examinations. From these results, mucociliary activity in the maxillary sinus, especially in the sinus ostium, rather than the direction of the mucociliary flow, is one of the most important factors in protecting against sinus infection.

L20 ANSWER 5 OF 6 BIOSIS COPYRIGHT 1996 BIOSIS DUPLICATE 3
AN 79:69617 BIOSIS
TI ISOLATION OF A **MESOGENIC NEWCASTLE DISEASE VIRUS** FROM AN ACUTE DISEASE IN INDONESIAN DUCKS.
SO TROP ANIM HEALTH PROD 10 (3). 1978 161-164 CODEN: TAHPAJ ISSN: 0049-4747
AU KINGSTON D J; DHARSANA R; CHAVEZ E R

L20 ANSWER 6 OF 6 CA COPYRIGHT 1996 ACS DUPLICATE 4
AN 80:130404 CA
TI Instability of hemagglutinin and neuraminidase in cells infected with different myxoviruses
SO Arch. Gesamte Virusforsch. (1973), 43(1-2), 98-102
CODEN: AGVIA3

Searched by: Mary Hale 308-4258

AU Gribkova, N. V.; Kaverin, N. V.; Tsvetkova, I. V.; Lipkind, M. A.
PY 1973
AB In chick embryo cells infected with a **m sog nic N wcastl dis as virus** strain (Beaudette), both hemagglutinin (HA) and neuraminidase (Nase) activities sharply decreased in response to cycloheximide, whereas after infection with velogenic strain no decrease was obsd. Orthomyxovirus Nase activity in chick embryo cells did not decrease in response to cycloheximide. HA activity decreased after cycloheximide treatment in cells infected with WSN and R5/I-influenza virus strains but not in fowl plague virus-infected cells. A possible relation between the degree of HA and Nase stability and the cleavage of myxovirus glycoproteins is discussed.